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MGMT promoter methylation testing to predict overall survival in people with glioblastoma treated with temozolomide: a comprehensive meta-analysis based on a Cochrane Systematic Review

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Abstract

Background. The DNA repair protein O^6-methylguanine-DNA methyltransferase (*MGMT*) causes resistance of tumor cells to alkylating agents. It is a predictive biomarker in high-grade gliomas treated with temozolomide, however, there is no consensus on which test method, methylation sites, and cutoff values to use.

Methods. We performed a Cochrane Review to examine studies using different techniques to measure *MGMT* and predict survival in glioblastoma patients treated with temozolomide. Eligible longitudinal studies included (i) adults with glioblastoma treated with temozolomide with or without radiotherapy, or surgery; (ii) where *MGMT* status was determined in tumor tissue, and assessed by 1 or more technique; and (iii) where overall survival was an outcome parameter, with sufficient information to estimate hazard ratios (HRs). Two or more methods were compared in 32 independent cohorts with 3474 patients.

Results. Methylation-specific PCR (MSP) and pyrosequencing (PSQ) techniques were more prognostic than immunohistochemistry for *MGMT* protein, and PSQ is a slightly better predictor than MSP.

Conclusions. We cannot draw strong conclusions about use of frozen tissue vs formalin-fixed paraffin-embedded in MSP and PSQ. Also, our meta-analysis does not provide strong evidence about the best CpG sites or threshold. MSP has been studied mainly for CpG sites 76-80 and 84-87 and PSQ at CpG sites ranging from 72 to 95. A cutoff threshold of 9% for CpG sites 74-78 performed better than higher thresholds of 28% or 29% in 2 of the 3 good-quality studies. About 190 studies were identified presenting HRs from survival analysis in patients in which *MGMT* methylation was measured by 1 technique only.

Key Points

- Largest meta-analysis of predictive value of *MGMT* test methods, cutoff methylated/unmethylated status, and CpG sites.
- Comparison of studies using 2 or more *MGMT* test methods.
- Methylation-specific PCR and pyrosequencing techniques best prognosticators.

Importance of the Study

It is important to reach a consensus of the best method(s) for assessing *MGMT* methylation status, based on the prognostic value of each method in predicting overall survival in people with glioblastoma treated with temozolomide. Currently, there is no consensus which CpG sites in the *MGMT* promoter region to be analyzed and which are the most relevant cutoffs to determine methylated vs unmethylated status for quantitative tests. Previous systematic reviews have assessed the prognostic value of *MGMT* promoter status assessed by a specific technique, for example, by pyrosequencing or methylation-specific PCR. However, no review has quantitatively determined which method correlates best with prognosis (although a previous study provided a narrative overview). In our Cochrane Review, we address a research priority question identified by the James Lind Alliance Neuro-Oncology Priority Setting Partnership—an organization joining academics, patients, carers, and clinicians to set research priorities in different fields.

The IDH (isocitrate dehydrogenase) wild-type glioblastoma (glioblastoma multiforme [GBM]) is the most common primary brain tumor in adults, with an annual incidence of approximately 3/100 000 population. The standard therapy is surgical resection followed by radiotherapy and adjuvant treatment with temozolomide, an alkylating agent. The median overall survival is 9.9 months for people treated with surgery plus radiotherapy and 15 months for people treated with surgery, radiotherapy, and chemotherapy.[1] For people with IDH-mutant glioblastomas, median overall survival is 24 months for people treated with surgery and radiotherapy, and 31 months for people treated with surgery, radiotherapy, and chemotherapy.[1] The cytotoxic effects of temozolomide are exerted by induction of O^6-methylguanine and are counteracted by the repair enzyme O^6-methylguanine-DNA methyltransferase (*MGMT*).[2] Expression of *MGMT* is highly regulated by epigenetic silencing of the *MGMT* gene promoter and thus the *MGMT* promoter methylation status is a widely used predictive marker for high-grade gliomas undergoing therapy with alkylating agents. However, *MGMT* methylation status does not always reflect gene expression, so the exact mechanism by which *MGMT* promoter methylation improves response to alkylating therapy is still unknown.

MGMT promoter methylation status testing is essential to inform treatment decisions in certain patients with GBM. For example, treating elderly patients with an unmethylated *MGMT* promoter with temozolomide has been shown to be detrimental when single-agent temozolomide chemotherapy was compared to radiotherapy.[3,4] On the basis of these findings, professional bodies, such as the European Association for Neuro-Oncology (EANO), recommend evaluation of *MGMT* promoter methylation status in elderly people,[5] and The National Institute for Health and Care Excellence (NICE) recommends that all high-grade gliomas are tested.[6] Most non-elderly (aged under 65 years) people are currently treated with temozolomide chemotherapy regardless of *MGMT* promoter status, due to the lack of alternative treatments.[7] However, *MGMT* promoter status is still a useful prognostic marker that may impact clinical management and may also be used for recruitment into clinical trials for novel therapies.

A number of methods have been established to assess *MGMT* promoter methylation status: methylation-specific PCR (MSP), quantitative (real-time) MSP, such as MethyLight MSP, pyrosequencing (PSQ), bead array, methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA)-PCR with high-resolution melting (HRM), co-amplification at lower denaturation temperature (COLD)-PCR, and digestion-based assays. Immunohistochemical detection of the *MGMT* protein or enzymatic activity has also been used as a proxy for methylation status. However, internationally accepted consensus about the most appropriate diagnostic method for *MGMT* promoter status is lacking.[8] MSP was used to assess *MGMT* promoter status in the landmark study by Hegi et al.[9] The choice of technique to assess *MGMT* promoter status in practice also depends on the amount and quality of the DNA sample(s) (eg, formalin-fixed paraffin-embedded (FFPE) vs frozen tissue-derived DNA), the robustness and simplicity of the method, the availability of equipment and reagents necessary for each of the techniques, cost, and experience. In the last United Kingdom National Quality Assessment (UK NEQAS) External Quality Assessment report, of 18 UK laboratories, 10 used PSQ, 5 MSP, 2 HRM, and 1 MS-MLPA. *MGMT* promoter methylation can also be determined with Illumina bead chip

arrays, an increasingly popular method for brain tumor classification based on the epigenetic profile.[10,11] All techniques can only interrogate methylation status in specific regions within the *MGMT* promoter, and the effect of methylation status at different sites on prognosis is not well understood. In addition, some of the techniques quantify the amount of methylation present, and there is no consensus regarding the cutoff for categorizing methylation status.

We undertook a Cochrane Review[12] to assess which way of measuring methylation of the *MGMT* promoter best predicts survival when people with glioblastoma are treated with temozolomide. The present article provides a summary of the key findings from the Cochrane Review.

Methods

Study Eligibility

Longitudinal studies of (i) adults (18 years and older) with (ii) first occurrence or recurrent glioblastoma, (iii) treated with temozolomide, and (iv) optionally concomitant and adjuvant therapies in addition to temozolomide, such as surgery or radiotherapy or both) (v) for whom the *MGMT* status was assessed by 1 or more techniques on tumor tissue, (vi) taken prior to treatment, but (vii) not in other types of samples such as blood samples, or by neuroimaging, were eligible for inclusion. Forms of glioma other than glioblastoma could be represented only if they constituted less than 10% of the total cases.

Eligible studies had to assess *MGMT* promoter methylation status in tumor tissue by 1 or more techniques. Eligible techniques included, but were not restricted to, (i) MSP; (ii) quantitative MSP (real-time PCR or MethyLight methylation-specific quantitative PCR); (iii) methylation-specific sequencing, including PSQ; (iv) bead array; (v) MS-MLPA; (vi) PCR with HRM; (vii) COLD-PCR; and (viii) digestion-based assays. We also included studies assessing (ix) *MGMT* expression (eg, immunohistochemistry [IHC] for protein expression, (x) mRNA levels, or (xi) *MGMT* enzymatic activity. Studies not reporting the test methods were excluded. Studies had to report a hazard ratio (HR), or data sufficient to allow a HR to be calculated. All techniques are listed in Table 1.

Search Methodology

Electronic searches were performed on the following databases up to December 2018: Ovid MEDLINE, PubMed (NOT MEDLINE), Ovid Embase, BIOSIS, and Web of Science Conference Proceedings Citation Index (CPCI-S). No restrictions were applied to language or date of publication. Other resources for searches were meeting abstracts from the Society of Neuro-Oncology (SNO), EANO, and the Japan Society for Neuro-Oncology (JSNO), retrieved via the CPCI-S. We examined the reference lists of included studies and of systematic reviews that have assessed the prognostic value of *MGMT* promoter status overall[43] or as assessed by a specific technique; for example, by PSQ[44] or MSP.[45]

Study Selection and Data Extraction

We used EPPI-Reviewer 4 (https://eppi.ioe.ac.uk) for processes of screening and selection of studies and for part of the data extraction the review.[46] Data were extracted and further analyzed in Microsoft Excel. Two review authors ("reviewers") independently screened titles and abstracts of all identified search results and determined whether full texts should be retrieved. Then, 2 reviewers independently assessed the full-text articles. Disagreements

Table 1 Summary of the Characteristics of the Included Studies Comparing 2 and More Techniques

Technique	Abbreviation	No. of Studies	References
Pyrosequencing	PSQ	20	13–27
Methylation-specific PCR	MSP	17	10,13,16–19,21–37
Immunohistochemistry	IHC	9	13,18–20,22,26,31,33,36,38,39
Quantitative MSP	qMSP	8	13,16,19,20,24,29,37,40
PCR with high-resolution melting	HRM-PCR	3	13,16,35
Bead array		2	10,41
PCR targeting mRNA	PCR-mRNA	2	20,30,38
Methylation-specific multiplex ligation-dependent probe amplification	MS-MLPA	1	34
Methylation-specific restriction enzyme quantitative PCR	MS-RE-qPCR	1	42
Methyl-beaming		1	42
Quantitative fluorescence immunohistochemistry	QF-IHC (AQUA)	1	29
Double immunofluorescence		1	NS cohort[15], RSD cohort[15]
qMSP combined with PSQ		1	22
qMSP combined with sequencing		1	27

Abbreviations: NS, Nordic Study; RSD, Region of Southern Denmark.

were resolved either by consensus or by consulting a third reviewer. A Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram was established (Figure 1) to describe the flow of information through the different phases of the review.

Full data extraction, risk-of-bias assessment, and synthesis were performed on studies that evaluated *MGMT* promoter methylation status using 2 or more methods, enabling comparisons of methods to be made using the same samples of patients. Two reviewers independently performed data extraction on each of these articles. Disagreements were resolved by consensus, and a third reviewer was consulted when necessary. Table 2 lists the items extracted.

We treated each method for determining *MGMT* promoter methylation status as a separate prognostic factor and extracted preferentially an unadjusted HR and its confidence interval (CI) for each method. Where unadjusted

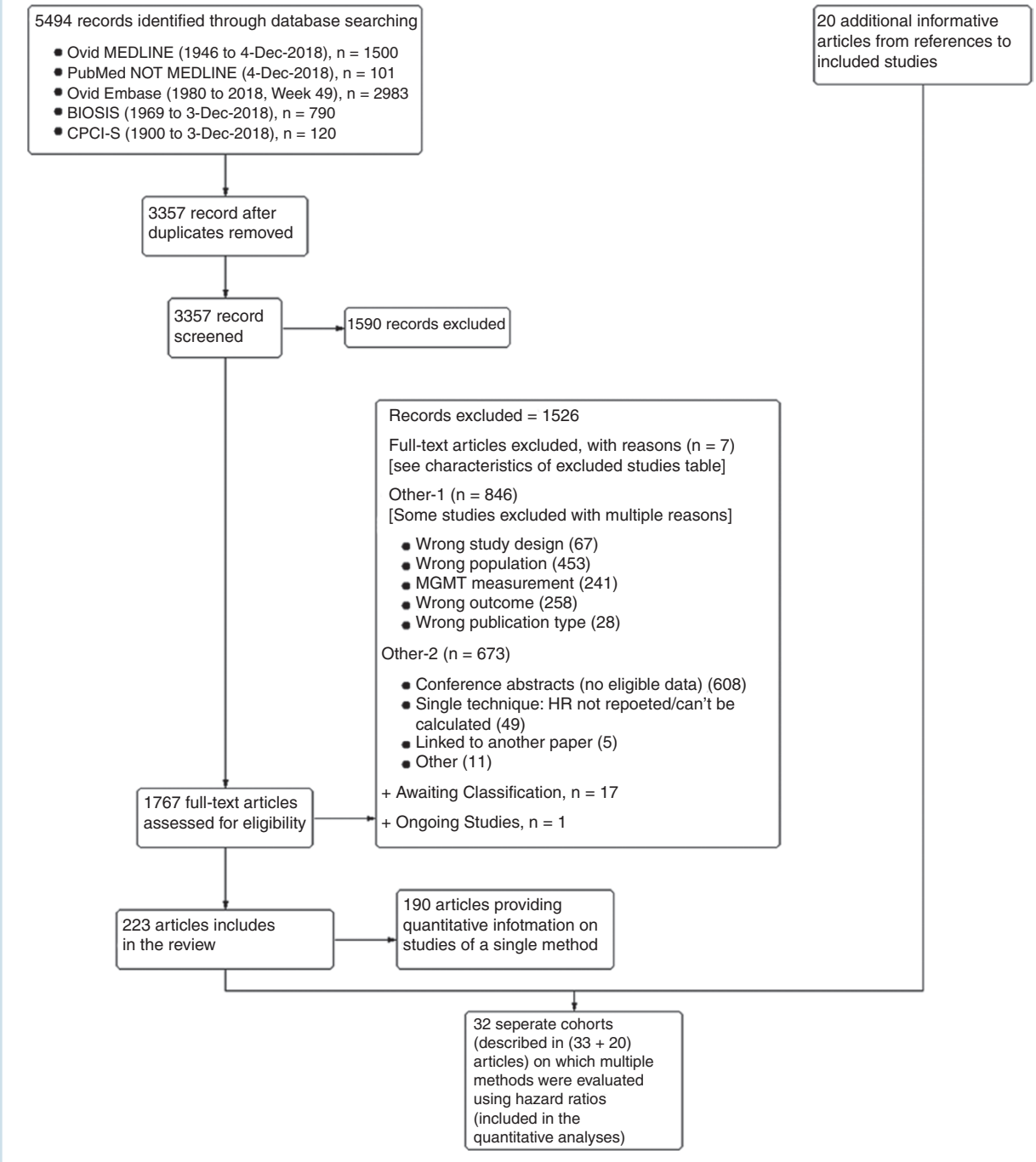

Fig. 1 Study flow diagram illustrating the selection process of records identified in the search.

Table 2 Parameters Captured and Assessed for Each Included Study of 2 or More Methods

Study characteristics	Author
	Year
	Country
	Length of follow-up
	Study dates
	Study design
Population characteristics	Number of participants
	Population source and setting
	Timing of *MGMT* promoter methylation assessment
	Inclusion/exclusion criteria
	Tumor type
	Age
	Gender
	Karnofsky performance status
	Extent of resection
	Treatment regimen
	Length of time between neurosurgery and start of treatment
	IDH mutation status
	First diagnosis or recurrent disease
	Deaths during follow-up
	Prevalence of *MGMT* promoter methylation (by each technique
Method(s) of *MGMT* promoter methylation assessment	Technique
	Tumor sample type (ie, FFPE or frozen tissue)
	Region/CpGs analyzed (for PCR-based tests); antibody used (for immunohistochemistry
	Cutoff/threshold used to determine *MGMT* promoter methylation status (where relevant)
Outcome assessment	Timepoint from which overall survival is measured
Missing data	Number of participants with any missing data

Abbreviations: FFPE, formalin-fixed paraffin-embedded; IDH, isocitrate dehydrogenase; *MGMT*, O^6-methylguanine-DNA methyltransferase.

HRs were not reported directly, we obtained them from reported individual participant data (IPD), reported adjusted HR's or reconstructed IPD from published Kaplan-Meier survival curves.[47] When IPD or reconstructed IPD available for 3 or more groups, the groups were combined to enable 2-way comparison (eg, by comparing "unmethylated" with combined "weakly methylated" and "methylated").

For studies that evaluated *MGMT* promoter methylation status using only a single method, a single reviewer extracted information on author, year, country, follow-up, number of participants, tumor type, IDH mutation status, and *MGMT* technique.

Assessment of Risk of Bias

The risk of bias in studies evaluating *MGMT* promoter methylation status of the same patients using at least 2 methods was assessed with a modified version of the Quality in Prognosis Studies (QUIPS) tool,[48] across the domains: study participation, subsequent treatment, outcome measurement, prognostic factor measurement, study attrition, adjustment for other potential prognostic factors (where relevant), and selective reporting.

Data Synthesis and Meta-Analysis

The prognostic ability of each individual method was quantified using a HR for overall survival, presented with a 95% CI. Comparisons of tests were restricted to those that could be made on the same patients within the same study. The directions of HRs were harmonized to reflect a better outcome with a greater HR. Where 5 or more studies had compared the same pair of techniques on the same patients, we computed ratio of hazard ratios (RHR), and combined these across studies using standard random-effects meta-analysis methods.[49] We evaluated certainty in the evidence following the GRADE framework.[50]

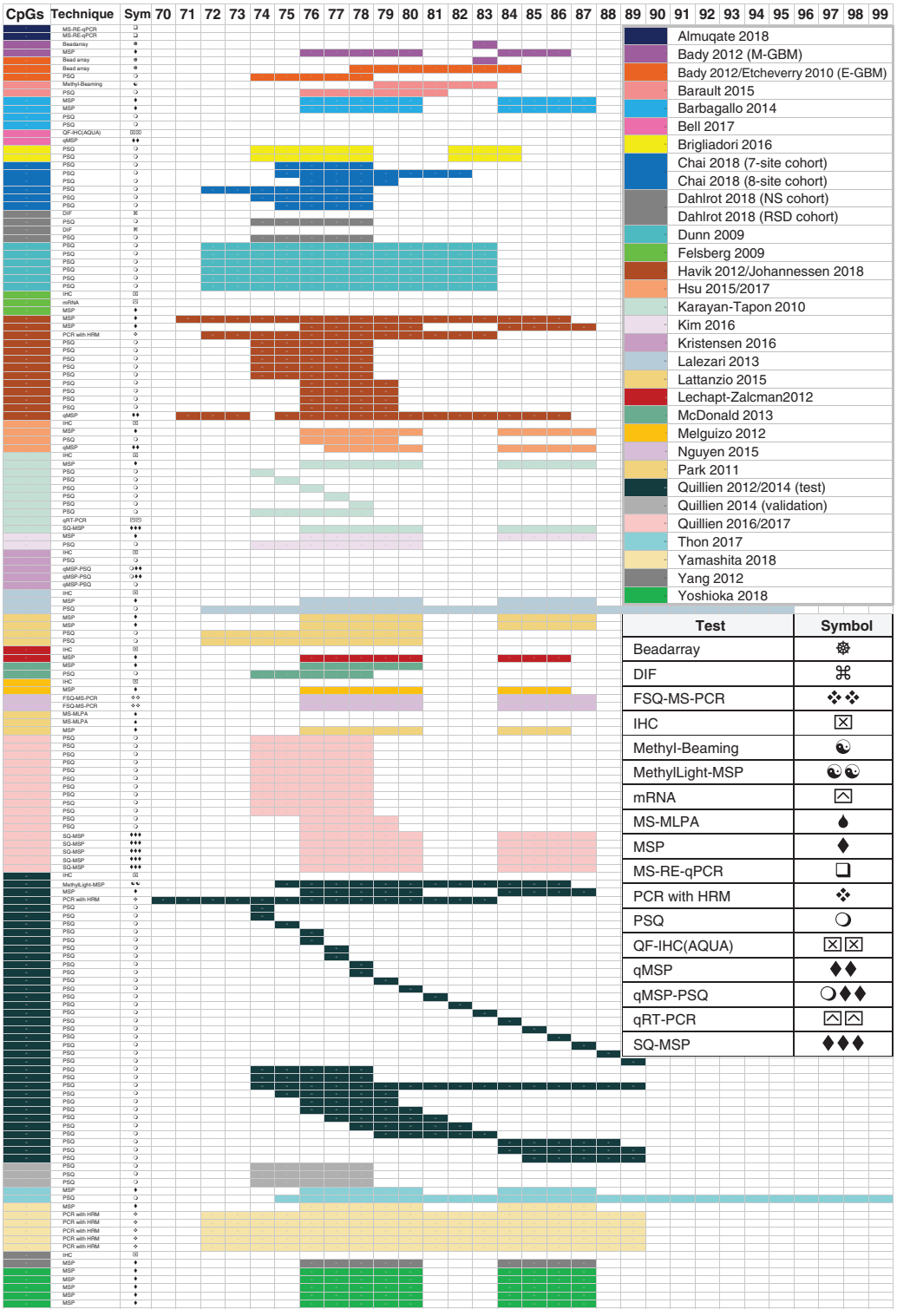

Fig. 2 Schematic overview of the CpG sites tested in the different publications. The first column is a color-coded representation of the authors, which are shown in the inset on the right. The CpG sites are listed in numerical order, corresponding to the iterative positions relative to transcription start. The corresponding sites, test methods, and thresholds are shown in detail in the Supplementary data. Each row represents a distinct method and where applicable, different CpG sites or thresholds. Rows with blank cells (ie, no color-coded CpG sites) indicate that a method was not PCR-based test or that CpG information is not available. For studies using PCR primers as described by Esteller et al.[51] CpG site location is based on Malley et al.[52]

Additional Analyses

The full Cochrane Review includes more details of the methods and further analyses including adjusted HRs (examining the prognostic value of tests in addition to age and extent of resection) and sensitivity analyses. In addition, it collates information about the UK costs of the main techniques and cost comparison ratios.[12]

Results

Results of the Search

The search identified 5494 records, of which 223 were included in the review (see Figure 1). These comprised 32 separate cohorts of patients ("studies") where 2 or more methods were compared, including studies comparing different variants of the same technique. About 190 further articles describing single-technique studies were also included and are described in a separate section below.

Characteristics of the Included Studies

The 32 studies included a total of 3474 participants. The techniques investigated and the corresponding references are listed in Table 1. All studies had a standard cohort design. Studies were undertaken in Europe (n = 19), North America (n = 2), East Asia (n = 8), Australia (n = 1), or in multiple countries (n = 2). Average patient age ranged from 44 to 64, with an overall male: female ratio of 1.5:1. The vast majority were patients with glioblastomas, predominantly undergoing total resection. Figure 2 illustrates the CpG sites targeted in the studies. The Supplementary data provide a comprehensive overview of the data from all individual comparison studies.

Findings: Comparisons of Different Techniques

The 160 extracted HRs are reported in the Supplementary data and summarized in Table 3. In all cases, the estimated HR is above 1, indicating higher hazard of death in those with unmethylated *MGMT* promoters. In the vast majority of cases, the lower limit of a 95% CI for the HR is above 1, confirming the prognostic value of *MGMT* promoter methylation status. When examining these results, we emphasize that comparisons should only be made of different methods within studies. HRs should not be compared across studies because there are many (more substantial) differences between these results than the choice of technique, tumor sample, CpG islands, or thresholds.

Meta-analysis of RHR (Table 3) shows that MSP (CpG sites 76-80 and 84-87) is more prognostic than IHC (varying thresholds) with RHR = 1.31 (95% CI: 1.01-1.71). Since a large majority of MSP studies had examined CpG sites 76-80 and 84-87,[52] we were unable to compare alternative CpG sites for MSP. We also found evidence that PSQ is

Table 3 Summary of Findings of Comparisons of Methods for Measuring *MGMT* Promoter Methylation Status

Technique 1	Technique 2	RHR (95% CI)	Participants	Studies	Certainty of Evidence	Reason for Down Rating
MSP	IHC	1.31 (1.01-1.71)	913	7	Moderate	Imprecision
PSQ	IHC	1.36 (1.01-1.84)	871	5	Low	Imprecision and indirectness (due to variability in CpG sites and thresholds used for PSQ)
PSQ	MSP	1.14 (0.87-1.48)	1119	9	Low	Imprecision and indirectness (due to variability in CpG sites and thresholds used for PSQ)
PSQ	PSQ (variant of)	Not estimated	876	11	Very low	Serious risk of bias, imprecision, inconsistency, and indirectness
qMSP	MSP of PSQ	Not estimated	765	7	Very low	Risk of bias, imprecision, inconsistency, and indirectness
Bead array	MSP of PSQ	Not estimated	81	2	Very low	Serious imprecision, inconsistency, and indirectness
PCR-mRNA	MSP or PSQ	Not estimated	148	2	Very low	Imprecision, inconsistency, and indirectness
MS-MLPA	MSP or PSQ	Not estimated	48	1	Very low	Serious risk of bias, serious imprecision, inconsistency, and indirectness
PCR-HRM	MSP or PSQ	Not estimated	309	3	Very low	Risk of bias, serious imprecision, inconsistency, and indirectness
Others	MSP or PSQ	Not estimated	1209	7	Very low	Serious imprecision, inconsistency, and indirectness

Abbreviations: CI, confidence interval; RHR, ratio of hazard ratios; for technique abbreviations, see Table 2.
The outcome being predicted is overall mortality (time to death). Grades of evidence: high quality, further research is very unlikely to change our confidence in the conclusion; moderate quality, further research is likely to have an important impact on our confidence in the conclusion; low quality, further research is very likely to have an important impact on our confidence in the conclusion; very low quality, we are very uncertain about the conclusion.

more prognostic than IHC (RHR = 1.36; 95% CI: 1.01-1.84), although studies of PSQ feeding into this analysis had targeted different CpG sites. While there is a consistent pattern that PSQ seems to be a slightly better predictor than MSP, there is no strong statistical evidence to confirm this (RHR = 1.14; 95% CI: 0.87-1.48). The CpG sites targeted by PSQ ranged between 72 and 95, and several studies had examined sites 74-78. There was a suggestion that PSQ (mainly at CpG sites 74-78, but with varying thresholds) is slightly more prognostic than MSP (sites 76-80 and 84-87).

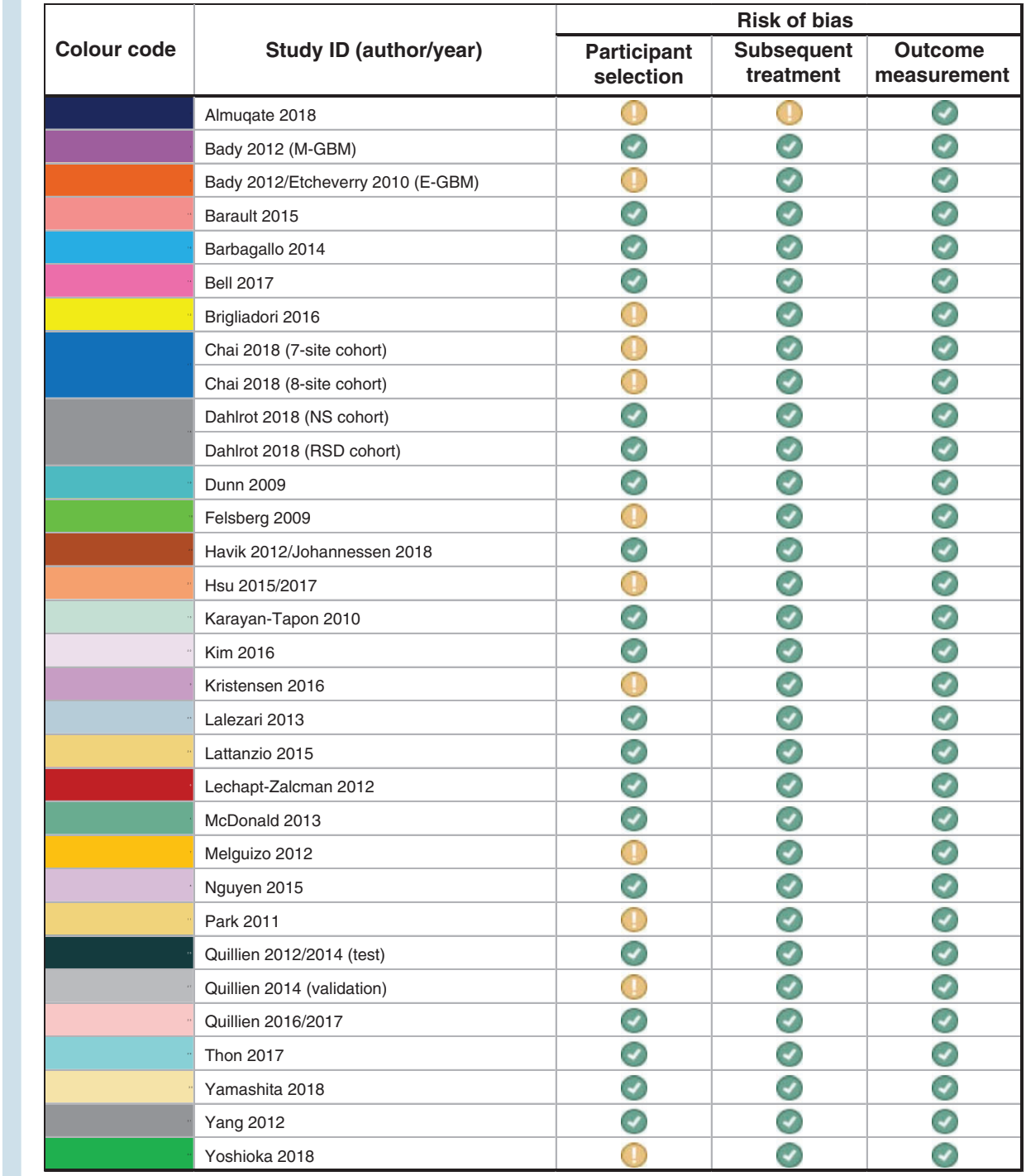

Fig. 3 Study-level risk-of-bias assessments for studies comparing 2 or more methods. participant selection, subsequent treatment, and outcome. Green (+) = low risk of bias; Yellow (–) = unclear risk of bias. The color codes of the individual studies correspond to those in Figure 1. Abbreviations: GBM, glioblastoma multiforme; NS, Nordic Study; RSD, Region of Southern Denmark.

We did not perform formal analyses to investigate whether heterogeneity in HRs may have been due to age, extent of tumor resection, Karnofsky performance status, IDH status, first diagnosis vs recurrence, start and length of follow-up, due to the very limited replication of specific methods, and large amounts of missing data for many of these study characteristics.

Many variants of PSQ have been compared, although we did not see any strong and consistent messages from the results. Thresholds varied substantially (from 4% to 25% for single CpG sites; and from 2.68% to 35% for multiple CpG sites). Two of the three studies with low (or unclear) risk of bias that compared different thresholds found that a 9% threshold was more prognostic than higher thresholds (of 28% or 29%; see top 2 results in Figure 4). We are unable to draw strong conclusions about use of frozen tissue vs FFPE in MSP, although 1 study observed that MSP was more prognostic when based on frozen tissue. No clear difference was apparent between using PSQ on FFPE vs frozen tissue.

Risk-of-Bias Assessment and Certainty in the Evidence

We present results of the risk-of-bias assessment for the 3 domains that apply to the whole studies in Figure 3. All studies were assessed to be at low or unclear risk of bias for participant selection. All studies except one were assessed as at low risk of bias arising due to variation in subsequent treatment after collection of the tumor sample. All studies were assessed to be at low risk of bias in measurement of the outcome (all-cause mortality). The other aspects of the risk-of-bias assessment apply to individual results. We were mostly free of concerns about risk of bias in the domains for study attrition, problems with other prognostic factors adjusted for, and selective reporting. For some results, the threshold used to classify methylation status was derived from the data, leading to a high risk of bias. The result-level risk-of-bias assessments for studies examining PSQ are included in Figure 4. Table 3 summarizes the certainty of the evidence from comparative studies, grouped by technique.

Table 4 Characteristics of Studies Examining *MGMT* Promoter Methylation With 1 Technique Only

Study Parameter	Characteristics	No. of Studies
Total number of studies		190
Reporting follow-up information		54
Reporting follow-up range		29
Reporting data on IDH1/IDH2 mutation		62
	All IDH wild type	11
	IDH mutation present (0.7%-73.4%)	47
	No IDH mutation reported	3
Reporting tumor type	Glioblastomas only (all studies)	183
	Glioblastoma: supratentorial	9
	Glioblastoma: primary	23
	Glioblastoma: primary, supratentorial	1
	Glioblastoma: recurrent	4
	Mixed glioma + gliosarcoma	6
	Gliosarcoma only	1
Test method	MSP	94
	PSQ	27
	qMSP (real-time PCR or MethyLight)	22
	Bead array	10
	MS-MLPA	4
	HRM-PCR	3
	MGMT protein (IHC)	21
	MGMT protein (Western blot)	1
	mRNA	4

Abbreviations: HRM-PCR, PCR with high-resolution melting; IDH, isocitrate dehydrogenase; IHC, immunohistochemistry; *MGMT*, O^6-methylguanine-DNA methyltransferase; MS-MLPA, methylation-specific multiplex ligation-dependent probe amplification; MSP, methylation-specific PCR; PSQ, pyrosequencing; qMSP, quantitative methylation-specific PCR.
As per the study protocol, the results of these studies were not examined, because comparisons of HRs across studies would not provide reliable indicators of differences between the methods.

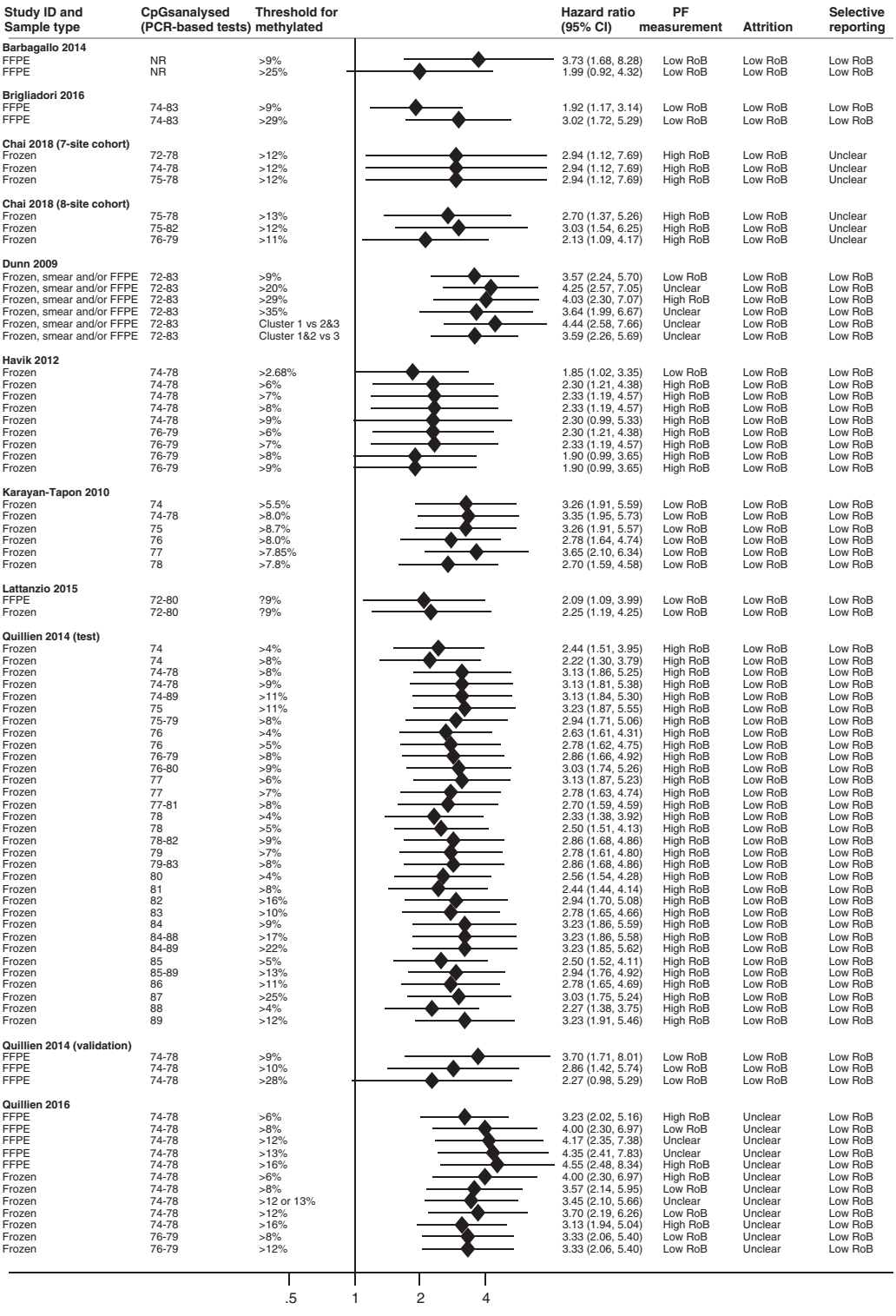

Fig. 4 Hazard ratios from studies comparing different methods for PSQ. Hazard ratios from studies comparing different methods for PSQ. The scale on the bottom of the figure indicates the hazard ratio. Abbreviations: CI, confidence interval; CpG, 5′-cytosine-phosphate-guanine-3′; FFPE, formalin-fixed paraffin-embedded; NR, not reported; PF, prognostic factor; PSQ, pyrosequencing; RoB, risk of bias.

Studies Examining Only a Single Technique

About 190 articles described studies presenting HRs from survival analysis in patients in which *MGMT* methylation was measured by 1 technique, and studies in which more than 1 technique was used but only *MGMT* methylation data from 1 method were used in the survival analysis (Table 4). These studies included a total of 27 710 participants (range 6-1395). They were conducted in Italy (n = 29), multiple countries (n = 23), Germany (n = 21), the United States (n = 20), Japan (n = 18), China (n = 17), South Korea (n = 11), France (n = 9), Denmark (n = 8), Spain (n = 8), the United Kingdom (n = 6), India (n = 3), Switzerland (n = 3), Australia, Belgium, Czech Republic, Egypt, Taiwan (n = 2), and 1 study each in Canada, Portugal, Netherlands, and Tunisia.

Discussion

We took a systematic approach to identifying, appraising, and collecting information from the evidence and assessed risk of bias and applicability concerns using a modification of QUIPS specific to the topic of the Cochrane Review.[48] This is the first systematic review to our knowledge that compares methods for categorizing tumors as methylated in relation to their ability to predict survival in patients with glioblastoma. Unsurprisingly, among methods for assessing *MGMT* status in glioblastoma patients treated with temozolomide, PSQ and MSP appear to be more prognostic for overall survival than IHC. While there is a consistent pattern that PSQ seems to be a slightly better predictor than MSP, there is no strong statistical evidence to confirm this. Moreover, there is no strong evidence to draw conclusions with confidence about the best CpG sites or thresholds for quantitative methods. In our study, MSP has been studied mainly for CpG sites 76-80 and 84-87 and PSQ at CpG sites ranging from 72 to 95. A cutoff threshold of 9% for CpG sites 74-78 was found to perform better than higher thresholds of 28% or 29% in 2 of the 3 good quality studies making such comparisons.[13,14,53]

To ensure fair comparison of methods, we assessed comparisons on the same patients and tumors within a study. A large variety of variants have been examined, particularly the use of different CpG sites and thresholds for PSQ, as well as a mixture of use of FFPE and frozen tumor samples. There was only a small amount of direct replicability across studies, meaning that firm conclusions were difficult to draw.

We limited eligibility for the review to studies that reported HRs or data sufficient for us to estimate them. In many instances, we reconstructed time-to-event data from Kaplan-Meier curves, allowing us to include 14 studies that we would not have included otherwise. However, there were still a small number of studies that had sought to compare methods but not presented data compatible with computation of HRs, which therefore did not meet our eligibility criteria.

We listed brief details of articles describing studies examining only 1 technique in the full Cochrane Review, although these were not included in the final meta-analysis (Table 4 and reference [12]). Among the studies that compared multiple techniques, we observed that HRs varied markedly across studies, and we were unwilling to make naive indirect comparisons of techniques across different studies and we are presenting quantitative results for these studies.[12]

We rated the evidence for the comparison between MSP and IHC as of "moderate certainty," and the evidence for comparisons of PSQ with MSP or IHC as of "low certainty" (Table 3). All other comparisons we rated as "very low certainty." Although risk-of-bias and publication bias were not major concerns for us, we rated down many of our assessments because there was a wide variety of different CpG sites and thresholds investigated, without systematic replications of findings using the same methods across studies. The amount of evidence is small, with only tens or at most the low hundreds of participants contributing to evidence for many of the techniques.

The evidence identified was generally applicable to clinical practice. We included only studies in which at least 90% of patients had glioblastoma, and nearly all patients were treated with temozolomide. We focused on overall survival only, so are unable to draw conclusions about using techniques to predict progression-free survival. The decision which method to use in clinical practice however is not necessarily guided by best predictive value but is influenced by cost, turnaround time, availability of equipment: PSQ, the most quantitative method can be limited by the availability of equipment, while qMSP, a commonly used method, cannot accurately quantify heterogeneously methylated CpG sites.

Further large studies examining the use of different techniques, using pre-defined threshold values for interpretation, would provide valuable new information on these methods, and our review reflects the reality that it may be challenging to reach a consensus for the best method of *MGMT* promoter methylation testing.

Supplementary Material

Supplementary material is available at *Neuro-Oncology* online.

Keywords

glioblastoma | meta-analysis | *MGMT* promoter methylation | prognostic biomarker | temozolomide

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